



UNIVERSITI PUTRA MALAYSIA

**ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC CONTENT
OFCOCOA (THEOBROMA CACAO L.) BEANS FROM
DIFFERENTCOUNTRIES**

AZIZAH BINTI OTHMAN

FPSK(M) 2005 13

3 SEP 2008

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COUNTRIES**

By

AZIZAH BINTI OTHMAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master of Science**

December 2005



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

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December 2005

Chairman : Amin Ismail, PhD

Faculty : Medicine and Health Sciences

This study was conducted to investigate the antioxidant capacity, phenolic and (-) epicatechin contents of cocoa beans from different countries, namely Malaysia, Ghana, Cote d'Ivoire and Sulawesi. A simple linear regression test was used to analyse the relationship between total phenolic and antioxidant capacity. Antioxidant capacity was assayed using four different assays namely, β -carotene bleaching, DPPH (2,2-diphenyl- β -picrylhydrazyl), ferric reducing/antioxidant potential (FRAP) and trolox equivalent antioxidant capacity (TEAC) methods. To estimate the content of total phenolic, an assay using Folin-Ciocalteu reagent was used. High-performance liquid chromatography (HPLC) was used to determine the (-) epicatechin content. In this study, two extraction media were used namely, ethanol and water. Between the two extracts, ethanolic extract of cocoa beans showed higher antioxidant capacity compared to water extract except for β -carotene bleaching assay. The antioxidant activity of

ethanolic extracts based on β -carotene bleaching method followed the order of Cote d'Ivoire > Malaysia > Ghana > Sulawesi. Water extract of Cote d'Ivoirian beans showed the highest antioxidant activity, followed by Ghana, Malaysia and Sulawesi with a significant difference at $p < 0.05$. All ethanolic extracts showed higher scavenging activity than that of water extract based on DPPH method. Ghanaian beans exhibited the highest scavenging activity, followed by Cote d'Ivoirian, Malaysian and Sulawesi. The scavenging activity of water extracts was in the order of Ghana > Malaysia > Cote d'Ivoire > Sulawesi. For FRAP method, Sulawesi beans had the highest antioxidant potential, followed by Malaysia, Ghana and Cote d'Ivoire. There was no significant difference between Sulawesi and Malaysian beans. The antioxidant potential of water extracts was in the order of Sulawesi > Ghana > Malaysia > Cote d'Ivoire. In TEAC assay, Sulawesi beans exhibited the highest antioxidant value for both ethanolic and water extracts, followed by Malaysia, Ghana and Cote d'Ivoire with a significant difference at $p < 0.05$. Malaysian beans showed significant highest value ($p < 0.05$) in phenolic content for both ethanolic and water extracts, followed by Sulawesi, Ghanaian and Cote d'Ivoirian. For epicatechin content, Sulawesi beans exhibited significant highest ($p < 0.05$) amount followed by Malaysian, Ghanaian and Cote d'Ivoirian. The results indicated that different assays revealed different antioxidant values. Moreover, the cocoa beans extracts from four different countries of origin showed different antioxidant capacities. A positive and high correlation were found between total phenolic and antioxidant potential (FRAP) for both ethanolic ($R = 0.764$) and water extracts ($R = 0.782$). While a positive moderate and low correlation were found between total phenolic content and TEAC for water and ethanolic extracts ($R = 0.685$, $R = 0.286$), respectively.

However, total phenolic content has negative correlation with antioxidant and scavenging activity. Antioxidant capacity of cocoa beans could be contributed by phenolic substances, through reducing potential. Moreover, (-) epicatechin content showed a positive and high correlation with antioxidant potential (ethanolic extracts, $R = 0.837$; water extracts, $R = 0.789$) and TEAC value (ethanolic extracts, $R = 0.918$; water extracts, $R = 0.895$) for both ethanolic and water extracts. Thus, indicating that, (-) epicatechin could be one of the phenolic contributes towards antioxidant capacity. Results indicated that antioxidant capacity, total phenolic and (-) epicatechin content of Malaysian beans were comparable to Ghanaian and Cote d'Ivoirian beans.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KAPASITI ANTIOKSIDAN DAN JUMLAH KANDUNGAN FENOLIK
BIJI KOKO (*THEOBROMA CACAO* L.) DARI PELBAGAI NEGARA**

Oleh

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Kajian ini dijalankan untuk mengkaji kapasiti antioksidan, jumlah kandungan fenolik dan (-) epikatekin biji koko dari pelbagai negara, iaitu Malaysia, Ghana, Cote d'Ivoire dan Sulawesi. Perkaitan di antara kandungan fenolik dan kapasiti antioksidan ditentukan dengan menggunakan ujian *simple linear regression*. Empat asai yang berbeza digunakan untuk menentukan kapasiti antioksidan iaitu asai pelunturan β -karotena, DPPH (2,2-difenil- β -pikrilhidrazil), penurunan ferik/kapasiti antioksidan (FRAP) dan kapasiti antioksidan kesetaraan troloks (TEAC). Kandungan fenolik ditentukan berdasarkan asai yang menggunakan reagen Folin-Ciocalteu telah digunakan bagi membuat anggaran jumlah kandungan fenolik. Kromatografi cecair bertekanan tinggi (HPLC) telah digunakan untuk menentukan kandungan (-) epikatekin. Dalam kajian ini, dua medium pengekstrakan iaitu etanol dan air digunakan. Di antara dua ekstrak ini, biji koko yang diekstrak menggunakan etanol menunjukkan kapasiti antioksidan yang tinggi kecuali, pada asai pelunturan β -karotena. Berdasarkan kaedah pelunturan β -karotena, aktiviti antioksidan adalah mengikut urutan Cote d'Ivoire > Malaysia

> Ghana > Sulawesi. Ekstrak air bagi biji koko Cote d'Ivoire menunjukkan aktiviti antiosidan yang paling tinggi, diikuti oleh Ghana, Malaysia dan Sulawesi dengan perbezaan yang signifikan pada $p < 0.05$. Berdasarkan kaedah DPPH, semua ekstrak etanol menunjukkan aktiviti penghapusan radikal bebas yang tinggi berbanding ekstrak air. Biji koko Ghana menunjukkan aktiviti penghapusan radikal bebas yang paling tinggi, diikuti oleh Cote d'Ivoire, Malaysia dan Sulawesi. Aktiviti penghapusan bagi ekstrak air adalah mengikut urutan Ghana > Malaysia > Cote d'Ivoire > Sulawesi. Bagi kaedah FRAP, biji koko Sulawesi mempunyai potensi antioksidan yang paling tinggi, diikuti oleh Malaysia, Ghana dan Cote d'Ivoire. Tiada perbezaan yang signifikan di antara biji koko Sulawesi dan Malaysia. Potensi antioksidan bagi ekstrak air adalah mengikut urutan Sulawesi > Ghana > Malaysia > Cote d'Ivoire. Biji koko Sulawesi menunjukkan nilai antioksidan yang paling tinggi bagi ekstrak etanol dan air berdasarkan asai TEAC, diikuti oleh Malaysia, Ghana, Cote d'Ivoire dengan perbezaan yang signifikan pada $p < 0.05$. Biji koko Malaysia menunjukkan kandungan fenolik paling tinggi ($p < 0.05$) bagi ekstrak etanol dan air, diikuti oleh Sulawesi, Ghana dan Cote d'Ivoire. Keputusan yang diperolehi menunjukkan asai yang berbeza memberikan nilai antioksidan yang berlainan. Lagipun, ekstrak biji koko yang berasal daripada empat negara yang berbeza menunjukkan kapasiti antioksidan yang berlainan. Didapati korelasi yang positif dan tinggi di antara jumlah fenolik dan potensi antioksidan (FRAP) bagi ekstrak etanol ($R = 0.764$) dan ekstrak air ($R = 0.782$). Sementara korelasi positif yang sederhana bagi ekstrak air ($R = 0.685$) dan rendah bagi ekstrak etanol ($R = 0.286$) telah diperolehi di antara jumlah fenolik dan nilai TEAC. Walau bagaimanapun, jumlah kandungan fenolik mempunyai korelasi yang negatif

dengan aktiviti antioksidan dan aktiviti penghapusan. Komponen fenolik biji koko melalui potensi penurunan berkemungkinan menyumbang kepada kapasiti antioksidan. Lagipun, kandungan (-) epikatekin juga menunjukkan korelasi positif dan tinggi bagi kedua-dua ekstrak dengan potensi antioksidan (ekstrak etanol, $R = 0.837$; ekstrak air, $R = 0.787$) dan nilai TEAC (ekstrak etanol, $R = 0.918$; ekstrak air, $R = 0.895$). Oleh itu (-) epikatekin mungkin merupakan salah satu komponen fenolik menyumbang kepada kapasiti antioksidan. Keputusan yang diperolehi menunjukkan kapasiti antioksidan, jumlah kandungan fenolik dan (-) epikatekin biji koko Malaysia setanding dengan biji koko Ghana dan Cote d'Ivoire.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, Dr. Amin Ismail for his tireless support, advice and guidance throughout the process of this study. My special thanks to my co-supervisors Dr. Ilham Adenan and Puan Nawalyah for their comment and advice.

I would like also to convey my appreciation to Puan Siti Muskinah for her help in HPLC analysis. To all lab assistants of Department of Nutrition and Health Sciences (UPM), your kindness will never be forgotten.

Last but not least, I would like to acknowledge my family and friends (you know who you are) for their endless support. My highest gratitude is dedicated to my husband for all his patience and love.

I certify that an Examination Committee has met on 27th December 2005 to conduct the final examination of Azizah Othman on her Master of Science thesis entitled “Antioxidant Capacity and Total Phenolic Content of Cocoa (*Theobroma cacao* L.) Beans From Different Countries” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



AZIZAH BINTI OTHMAN

Date: **0 5 JUN 2006**

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CHAPTER 1

INTRODUCTION

1.1 Background

Traditionally, major nutrients such as proteins, carbohydrates, minerals and vitamins are important for maintaining a good health. Healthy lifestyle and balanced diet could reduce the risk of chronic diseases such as cardiovascular diseases, diabetes and certain cancers. The relationship between diet and chronic diseases is well documented. Poor diet combined with lacking in exercise is the second leading cause of death in the United State (Papaz, 1999). Recently, diets containing antioxidants have received more attention because of their beneficial effect towards health.

Antioxidant components are micro constituents present in the diet that are involved in scavenging free radicals. Food contains a wide variety of antioxidant components such as flavonoids, carotenoids and certain vitamins. Epidemiological studies showed a negative correlation between the intake of antioxidant components in form of whole foods and certain chronic diseases (Acheson and Williams, 1983; Verlangieri *et al.*, 1985). To support a protective effect of flavonoids, a study among elderly males Dutch revealed that coronary heart disease is inversely correlated with the intake of flavonoids (Hertog *et al.*, 1993). Tea, wine, onion, grapes, tomatoes, spinach and apples are some dietary sources of flavanoids (Rice-Evans *et al.*, 1996). Besides vegetables, fruits and grains, few studies demonstrated that the consumption of cocoa or chocolate



reduced the risk of cardiovascular disease (Keen, 2001; Osakabe *et al.*, 1998). According to the Mayan and Mexican religions, cocoa had divine origins, which discovered by the gods in a mountain that also contained other delectable foods to be used by the Maya. Evidence for the use of cocoa and chocolate for medicinal purposes started from ancient Mexica (Aztecs) history. Chocolate was drunk by the Mexican to treat stomach and intestinal complaints. Cocoa beans was also reported to treat childhood diarrhea, to relieve fever and faintness (Dillinger *et al.*, 2000)

According to Duke (2003), two spoons of cocoa in a cup of water or milk could be used as a palliative treatment of Parkinson's disease, mastitis, liver diseases, sexual dysfunction, fever, cystitis, cold, burns, asthma and bronchitis, diabetes and obesity. Amin *et al.* (2004) showed that Malaysian cocoa liquor exhibited a potential in decreasing the severity of hepatocarcinogenesis in rats. Furthermore, Malaysian cocoa beans and cocoa powder have been found to have antihyperglycaemic effects on diabetic rats (Amin *et al.*, 2004; Ruzaidi *et al.*, 2005).

Polyphenols have received considerable attention because of their physiological functions including antioxidants, antimutagenic and anticancer activities. They could also impair the promotion stage of carcinogenesis as a result of their antioxidant activity (Vinson *et al.*, 1998). Besides tea and coffee, cocoa beans and its products (cocoa liquor, cocoa powder, and dark chocolate) are the most commonly consumed foods rich in polyphenolic compounds. Coffee and tea are regularly consumed as a beverage, while a large proportion of the cocoa

produced is consumed as solid chocolate, which has high nutritional and energy content.

Unfermented cocoa beans is in high phenolic content (12-18% dry weight) (Kim and Keeney, 1984). Dreosti (2000) reported that 60% of the total phenolic in raw cocoa beans are flavanol monomers (epicatechin and catechin) and procyanidins oligomers (dimer to decamer). These compounds were reported to be a potential candidate to combat free radicals, which are harmful to our body and food systems (Sanbogi *et al.*, 1998; Adamson *et al.*, 1999). *In-vitro* studies demonstrated that these compounds have several biological activities such as the ability scavenge to superoxide and hydroxyl radicals, reduce lipid peroxy radicals and inhibit lipid peroxidation (Kanner *et al.*, 1994; Salah *et al.*, 1995; Vinson and Hontz, 1995). In addition, it has been reported that polyphenols are incorporated into LDL particles and thereby decreasing their ability for being oxidised when isolated from plasma (Aviram and Fuhrman, 2003).

According to International Cocoa Organization (ICCO) world production of cocoa beans in 2002/03 was 3,102,000 tons, representing an increase of 241,000 tons (8%) from the level of 2,861,000 tons achieved in the 2001/02 season. The overall increase in world production of cocoa beans reflected an increase of 207,000 tons in Africa and 45,000 tons in the Americas. These increases were partially offset by a reduction of 11,000 tons in Asia (Malaysia and Indonesia). In Malaysia, Sabah has the biggest area planted cocoa tree in Malaysia, followed by Perak, Selangor, Pahang, Terengganu and Johor. In 1988, Malaysia is the fourth largest producer of cocoa beans in the world after Cote d'Ivoire, Brazil

and Ghana (Othman, 1993). However, according to ICCO (2002/2003), Malaysia is the ninth largest producer of cocoa beans. A total export of Malaysian dried cocoa beans was increased from 2400 tons (1970) to 157 000 tons (1987) which worth about RM684 million (MARDI, 1990). A total export of dried cocoa beans is more than RM800 million with price of RM3.18 per kg (FAMA, 1988). Moreover, Malaysian beans are sold at low price due to their poor flavour quality compared to Ghanaian and West African beans. There are some qualities weaknesses in Malaysian cocoa beans such as high acidic, small size of beans and thick shell compared to Ghanaian beans (MARDI, 1990). A study done by Jinap *et al.* (1995) showed, Malaysian cocoa beans had high percentage of bitterness and burnt flavour, which is lead to low response of strong chocolate flavour when compared to Ghanaian beans.

1.2 Statement of Problems

Cocoa (*Theobroma cacao* L.) was reported rich in polyphenols (Porter *et al.*, 1991). Its beans contained a lot of phenolic substances, about 12-18% (dry weight) of which, are present in unfermented beans. Cocoa beans and its product (cocoa powder and cocoa liquor) contain these substances, which been reported to have antioxidant properties (Osakabe *et al.*, 1998). Although cocoa beans have undergone primary and secondary processing, their phenolic compounds are still high.

A study done by Jinap *et al.* (1995) reported that Ghanaian and Nigerian cocoa beans yield the best chocolate flavour. Flavour quality of chocolate usually depends on the sources of the cocoa beans. Beans from different origins have been reported to have distinct flavour characteristics such as acidic, hammy or smoky (Powell, 1983). West African beans had a strong chocolate flavour, whereas high acidic beans produced by Malaysia and Indonesia have many objectionable flavours, such as, hammy, burnt and musty (Jinap *et al.*, 1995). The amount of (-) epicatechin in commercial beans from Brazil and Cote d'Ivoire was lower compared to beans from Costa Rica and Samoa due to fermentation practices (Kim and Keeney, 1984).

In this study, two extraction media (ethanol and water) were used for preparing the cocoa extracts. Previous study had reported that the properties of antioxidant activity and the yield of phenolic content influenced by different extracting solvents (Sun and Ho, 2005).

Ghanaian beans had a good flavour (taste and aroma) compared to Malaysian beans. No data to date have been published regarding antioxidant capacity of cocoa beans from different countries of origin. Natsume *et al.* (2000) reported that polyphenol content in cocoa liquor varied depending on country of origins. If there is no significant difference in term of antioxidant capacity between Malaysian and Ghanaian and Cote d'Ivoirian beans, Malaysian beans have a similar antioxidant capacity as compared to the standard (Ghanaian beans). This study would give better insight to the benefit of cocoa and chocolate, since people in around the world consume cocoa-based products as for foods or beverages in their daily diet.

1.3 Significance of Study

Currently, natural antioxidant has received more attention and to be part of daily diet. Cocoa beans is one of the main sources of polyphenols especially (-) epicatechin. (-) Epicatechin was reported to have antioxidant capacity (Kris-Etherton and Keen, 2002; Lee *et al.*, 2003). Antioxidant capacity is known as additional information besides their phenolic content. The quality of cocoa beans was determined based on their flavour characteristics. Numerous investigations are currently focused on the biological effect of cocoa and its flavanol and oligomer components. For example, cocoa had been reported enable to increase the antioxidant capacity and slow the oxidation rate of LDL (Waterhouse *et al.*, 1996; Kondo *et al.*, 1996; Sanbogi *et al.*, 1998). Thus, antioxidant capacity should be considered as one of the important parameters in order to determine the quality of cocoa beans.

There are several methods for antioxidant determinations and each has its own limitations. Therefore, four different antioxidant assays were used in this study. Antioxidant capacity of cocoa extracts was based on different antioxidant assay. It showed that, different assay could reveal different antioxidant activities. Results of this study would be useful for people to consume cocoa products not only as a part of diet, but also as a functional food towards reducing the risk for some diseases. Thus, it will increase the market and use of Malaysian cocoa by industry due to their contribution towards health benefits. We also want to investigate the antioxidant properties of different beans, and compared with Malaysian ones. We noticed that West African beans especially from Ghana and

Cote d'Ivoire have been recognised as standard beans (high flavour) compared to other countries including Malaysia. Thus, in this study, the investigation of antioxidant capacity and phenolic content of different beans from Malaysia, Indonesia, Ghana and Cote d'Ivoire was compared.

1.4 Objectives of Study

A general objective of this study was to investigate the antioxidant capacity of cocoa beans (*Theobroma cacao L.*) from different countries of origin namely Malaysia, Ghana, Cote d'Ivoire and Sulawesi (Indonesia).

The specific objectives of this study were:

1. To determine the antioxidant capacity of cocoa extracts from cocoa beans using four different assays.
2. To measure total phenolic and (-) epicatechin contents of cocoa extracts.
3. To measure the relationship between antioxidant capacity and total phenolic.
4. To compare the antioxidant properties of cocoa beans between water and ethanolic extracts.